Regulated Alternative Translocation: A Mechanism Regulating Transmembrane Proteins Through Topological Inversion

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Abstract

Transmembrane proteins must adopt a proper topology to execute their functions. In mammalian cells, a transmembrane protein is believed to adopt a fixed topology. This assumption has been challenged by recent reports that ceramide or related sphingolipids regulate some transmembrane proteins by inverting their topology. Ceramide inverts the topology of certain newly synthesized polytopic transmembrane proteins by altering the direction through which their first transmembrane helices are translocated across membranes. Thus, this regulatory mechanism has been designated as Regulated Alternative Translocation (RAT). The physiological importance of this topological regulation has been demonstrated by the finding that ceramide-induced RAT of TM4SF20 (Transmembrane 4 L6 family member 20) is crucial for the effectiveness of doxorubicin-based chemotherapy, and that dihydroceramide-induced RAT of CCR5 (C-C chemokine receptor type 5), a G protein-coupled receptor, is required for lipopolysaccharide (LPS) to inhibit chemotaxis of macrophages. These observations suggest that topological inversion through RAT could be an emerging mechanism to regulate transmembrane proteins.

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Abbreviations

CCR5	C-C chemokine receptor 5
CREB3L1	cAMP responsive element binding protein 3-like 1
ER	endoplasmic reticulum
GPCR	G protein-coupled receptor
LPS	lipopolysaccharide
RAT	regulated alternative translocation
RIP	regulated intramembrane proteolysis
TM4SF20	Transmembrane 4 L6 family member 20
TRAM	Translocating chain-associated membrane protein

1   Introduction

Cellular membranes are physical barriers that are impermeable to many molecules. Thus, unlike cytosolic and nuclear proteins that are surrounded by homogenous environment, transmembrane proteins are all polarized, as segments of

transmembrane proteins localized at different sides of membranes are exposed to distinct environment. As a result, the topology of transmembrane proteins, which depicts their orientation across membranes, is critical for their functions.

The topology of transmembrane proteins is primarily determined during their synthesis on endoplasmic reticulum (ER) membranes (Zimmermann et al. 2011). For transmembrane proteins that contain a single transmembrane helix, the translocation process has been categorized into three classes (Fig. 1) (Lodish et al. 2007). Type I insertion refers to proteins that contain a cleavable ER-targeting signal peptide N-terminal to the first transmembrane helix. The signal recognition particle binds to the hydrophobic sequence within a nascent signal peptide, directing the ribosome/nascent polypeptide complex to the ER membranes. The signal peptide is then inserted into membranes adjacent to the Sec61 ER translocon through a direction where its N-terminus faces cytosol. This insertion opens the lateral gate of the translocon, enabling

hydrophilic sequence C-terminal to the signal peptide to be transported into ER lumen (Rapoport et al. 2017). Following the translocation, the signal peptide is removed from the mature protein by the signal peptidase, causing the N-terminus of the mature protein to be embedded into ER lumen (Fig. 1a). The other two insertions do not use a signal peptide. Instead, their insertions are initiated by recognition of the hydrophobic sequence present in the transmembrane helix of nascent peptides by the signal recognition particle, which directs the nascent peptide/ribosome complex to the Sec61 ER translocon. In Type II insertion, the direction through which the transmembrane helix is inserted into membranes is the same as that of the signal peptide in Type I insertion, allowing sequence C-terminal to the transmembrane helix to be imported into ER lumen (Rapoport et al. 2017). As a result, the N-terminus of the Type II transmembrane proteins is located in cytosol (Fig. 1b). In Type III insertion, the transmembrane helix is inserted through an opposite
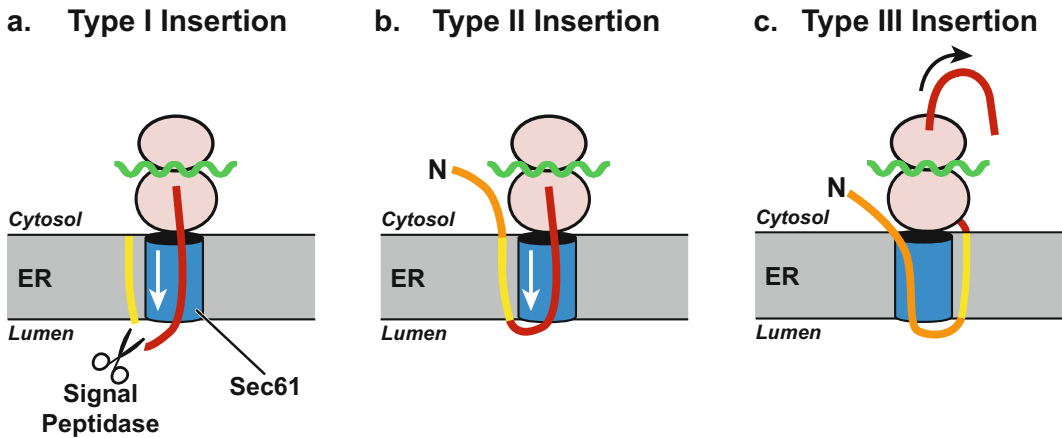

Fig. 1 Three classes of membrane translocation process

(**a**) During Type I insertion, the signal peptide is inserted into membranes with its N-terminus facing cytosol before it is removed from the mature protein by the signal peptidase. This insertion allows nascent peptides C-terminal to the signal peptide to be pushed through the Sec61 translocon by ribosomes. (**b**) Type II insertion is similar to the Type I insertion except that the translocation process is initiated by the transmembrane helix that cannot be cleaved by the signal peptidase. (**c**) During Type III

insertion, the transmembrane helix is inserted into membranes with the N-terminus embedded into the ER lumen. This insertion enables peptides N-terminal to the transmembrane helix to be pulled through the translocation channel. (**a–c**) The sequence N-terminal to the transmembrane helix, the signal peptide (**a**) or the transmembrane helix (**b**, **c**), and the sequence C-terminal to the transmembrane helix are highlighted in orange, yellow, and red, respectively. The arrow indicates the direction of nascent peptide elongation during translation

direction so that its N-terminus faces the ER lumen. This insertion enables hydrophilic sequence N-terminal to the transmembrane helix to be transported into the ER lumen (Rapoport et al. 2017). Following this type of insertion, the N-terminus of the proteins is located in the ER lumen (Fig. 1c).

For polytopic transmembrane proteins, their topology is determined by the direction through which the first transmembrane helix is inserted into membranes, which follows one of the three types of the insertion illustrated above (Rapoport et al. 2017). In bacteria, the transmembrane helices in polytopic membrane proteins can be reoriented co-translationally or post-translationally upon alterations in phospholipid contents in membranes (Bogdanov and Dowhan 1998; Bogdanov et al. 2002, 2014; Dowhan et al. 2019). However, this reposition of transmembrane helices has not been extensively reported for mammalian transmembrane proteins except for a few proteins such as aquaporin 1 and CD38 (Bogdanov et al. 2018; Lee and Zhao 2019; Lu et al. 2000). Recently, there are several reports demonstrating that topology of some mammalian transmembrane proteins can be reversed under certain physiological conditions, and this topological inversion is critical to regulate functions of these transmembrane proteins.

2 Topological Inversion of TM4SF20: A Critical Step in Doxorubicin-Based Chemotherapy

Doxorubicin, a chemotherapeutic reagent extensively used to treat various cancers, is believed to inhibit cancer cell proliferation through DNA damage by inhibiting DNA topoisomerase II (Gewirtz 1999; Yang et al. 2014). However, recent studies suggest that activation of a transcription factor called cAMP responsive element binding protein 3-like 1 (CREB3L1) is also critical for the cytostatic activity of doxorubicin (Denard et al. 2012; Patel and Kaufmann 2012). Unlike typical transcription factors, CREB3L1 is synthesized as a transmembrane precursor that contains a cytosolic N-terminal domain capable of functioning as a transcription factor (Denard et al. 2011; Murakami et al. 2006) (Fig. 2). In resting cells, CREB3L1 remains as the inactive transmembrane precursor. Doxorubicin enhances production of ceramide, which in turn triggers proteolytic activation of CREB3L1 through a pathway called Regulated Intramembrane Proteolysis (RIP) (Brown et al. 2000; Ye 2013). This pathway activates CREB3L1 through two proteolytic events: The first cleavage in the luminal domain catalyzed by Site-1 protease followed by
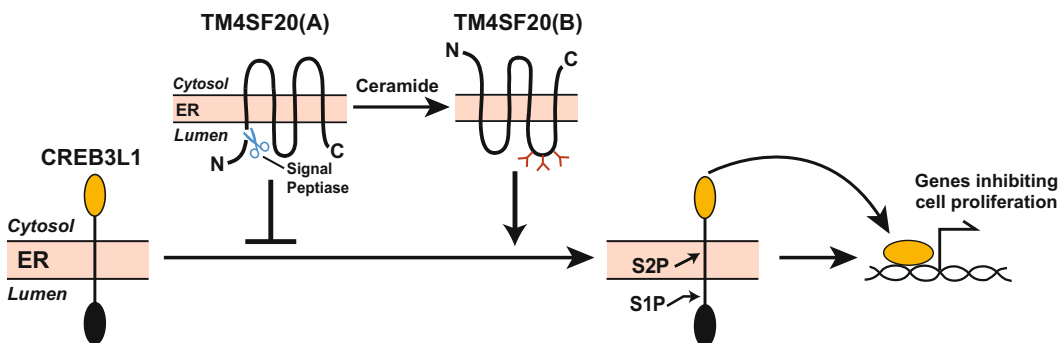

Fig. 2 RIP of CREB3L1 activated by RAT of TM4SF20
In the absence of ceramide, TM4SF20 adopts a topology (TM4SF20(A)) that inhibits proteolytic activation CREB3L1. Ceramide inverts the topology of newly synthesized TM4SF20 through RAT. The protein with the inverted topology (TM4SF20(B)) activates RIP of CREB3L1 catalyzed by Site-1 protease (S1P) and Site-2 protease (S2P). These cleavages release the N-terminal domain of CREB3L1 from membranes, allowing it to activate genes inhibiting cell proliferation

the second cleavage at the interface between the transmembrane helix and the cytosolic domain (Fig. 2). The final cleavage catalyzed by Site-2-protease releases the N-terminal domain of CREB3L1 from membranes, allowing it to activate transcription of genes such as p21 that inhibit cell proliferation (Cui et al. 2016; Denard et al. 2011, 2012) (Fig. 2). The importance of this CREB3L1-mediated pathway was illustrated by observations that at clinically relevant doses, doxorubicin was much more effective in cancer cells that expressed high levels of *CREB3L1* than in those expressing low levels of the gene. This correlation was documented in cancer cells cultured in vitro, in xenograft tumors established in mice, and in human tumor samples (Denard et al. 2012, 2015, 2018; Xiao et al. 2019). Thus, ceramide-induced RIP of CREB3L1 plays a critical role in doxorubicin-based cancer chemotherapy.

Later studies identified Transmembrane 4 L6 family member 20 (TM4SF20) as a polytopic transmembrane protein crucial for ceramide to induce RIP of CREB3L1. In the absence of ceramide, the N-terminus of TM4SF20 is inserted into the ER lumen, and the sequence N-terminal to the first transmembrane helix is removed co-translationally by the signal peptidase (Fig. 2). Under this configuration, the loop between the third and fourth transmembrane helix that contains three potential sites for N-linked glycosylation is located in the cytosol so that glycosylation cannot occur, and the C-terminus of the protein is located within the lumen as demonstrated by the protease protection assay (Fig. 2) (Chen et al. 2016). This form of the protein, which is designated as TM4SF20(A), inhibits RIP of CREB3L1 (Fig. 2) (Chen et al. 2014). Treatments of cells with exogenous ceramide or bacterial sphingomyelinase and doxorubicin that stimulates endogenous production of ceramide inverted the topology of TM4SF20 (Chen et al. 2016). In these cells, the N and C-termini of newly synthesized TM4SF20 are all located in the cytosol as demonstrated by the protease protection assay, and the loop containing the potential glycosylation sites is located in the ER lumen where it is glycosylated (Fig. 2) (Chen et al. 2016). This form of the protein, which is designated as TM4SF20(B), activates RIP of CREB3L1 (Chen et al. 2016). Thus, the ceramide-induced topological inversion turns TM4SF20 from an inhibitor to an activator for proteolytic activation of CREB3L1 (Fig. 2). Importantly, ceramide does not flip the topology of pre-existing TM4SF20 but inverts the topology of the newly synthesized protein, as treatment of cycloheximide, an inhibitor of protein synthesis, blocked ceramide-induced production of TM4SF20(B) (Chen et al. 2016). Therefore, ceramide appears to invert the topology of TM4SF20 by altering the direction through which it is translocated across membranes during its synthesis. This regulatory mechanism was thus designated as Regulated Alternative Translocation (RAT).

Since the N-terminal sequence of TM4SF20 (A) produced in the absence of ceramide is cleaved off from the mature protein by the signal peptidase, it is tempting to conclude that the first transmembrane helix of TM4SF20(A) is translocated through the Type I insertion, the mechanism responsible for insertion of proteins with an N-terminal signal peptide. However, several lines of evidence argue against this conclusion: First, the N-terminal sequence of TM4SF20 does not contain any hydrophobic residues characteristic for a signal peptide (Petersen et al. 2011); Second, the N-terminal sequence of TM4SF20 did not function as a signal peptide when it was fused to another protein (Chen et al. 2016); Third, the N-terminal sequence of TM4SF20 could be replaced by other peptides without altering the topology of TM4SF20 (A) and ceramide-induced RAT of TM4SF20 (Chen et al. 2016). Thus, even though the N-terminal sequence of TM4SF20(A) is cleaved off from the mature protein by signal peptidase, there is no evidence to suggest that the sequence actually functions as a signal peptide. It is more likely that this cleavage is caused by the accessibility of the peptide to signal peptidase in the ER lumen. This type of proteolysis by signal peptidase has been reported for processing of hepatitis C virus polyprotein in which the protease cleaves the protein at multiple sites in the ER lumen distal to the N-terminal sequence (Hijikata et al. 1991).

Since TM4SF20(A) does not contain a functional signal peptide, the first transmembrane helix of the protein is translocated across ER membranes through the Type III insertion. In contrast, the first transmembrane helix of TM4SF20(B) produced in the presence of ceramide is translocated through the Type II insertion. Thus, ceramide-induced RAT of TM4SF20 shifts the translocation of the first transmembrane helix of TM4SF20 from the Type III to Type II insertion. Since both of these insertions are initiated by interaction of the first transmembrane helix with the ER translocon, the first transmembrane helix of TM4SF20 may be critical for the topological regulation through RAT. Indeed, replacing the signal peptide of alkaline phosphatase with the N-terminal domain of TM4SF20 that contains the first transmembrane helix of the protein led to ceramide-induced RAT of the fusion protein (Chen et al. 2016). Further analysis revealed that a GXXXN motif present in the first transmembrane helix of TM4SF20 is critical for RAT of the protein, as mutating the Gly or Asn residue in the motif to Leu locked TM4SF20 in the reversed orientation (TM4SF20(B)) regardless of the presence of ceramide (Chen et al. 2016; Wang et al. 2019). These results suggest that in the absence of ceramide, the GXXXN motif present in the nascent peptide may interact with a component of the translocation machinery, allowing the first transmembrane helix to be translocated through the Type III insertion to produce TM4SF20(A).

Translocating chain-associated membrane protein 2 (TRAM2) may be such a translocation component involved in RAT of TM4SF20. TRAM2, a poorly characterized protein, is highly homologous to TRAM1, an accessory protein for the Sec61 translocation channel (Do et al. 1996; Görlich et al. 1992; Voigt et al. 1996). Knockdown of TRAM2 but not TRAM1 through RNAi facilitated production of TM4SF20(B) even in the absence of ceramide (Chen et al. 2016). This observation suggests that in the absence of ceramide, TRAM2 may interact with the GXXXN motif present in the first transmembrane helix of nascent TM4SF20 to enable the type III insertion of the transmembrane helix. Interestingly,

TRAM2, and all TRAM protein, contains a TLC domain that is postulated to bind ceramide or related sphingolipids (Winter and Ponting 2002). It will be interesting to determine whether ceramide regulates translocation of TM4SF20 through its interaction with TRAM2.

3 Topological Inversion of CCR5: A Critical Step for LPS to Inhibit Chemotaxis

A bioinformatics analysis revealed that ~100 proteins contain in their first transmembrane helix a GXXXN motif that is critical for RAT of TM4SF20. Remarkably, more than ~90% of these proteins are G protein-coupled receptors (GPCRs) (Denard et al. 2019). Interestingly, the first transmembrane helix of the majority of GPCRs is inserted into membranes through the Type III orientation (Guan et al. 1992; Von Heijne 2006). Since RAT alters the translocation of the first transmembrane helix of TM4SF20 from the Type III to Type II insertion, these observations raise the possibility that these GPCRs may also subject to ceramide-induced topological inversion through RAT.

One of these GPCRs, namely C-C chemokine receptor 5 (CCR5), indeed subjects to ceramide-induced topological inversion. In unstimulated macrophages, CCR5 adopts a configuration consistent with that of GPCRs. The N- and C-terminus of the protein with this topology, which was designated as CCR5(A), is localized at extracellular space and cytosol, respectively (Fig. 3). Under this configuration, CCR5 functions as a chemokine receptor, directing macrophages migrating toward its ligand, CCL5 (Denard et al. 2019; Duma et al. 2007; Oppermann 2004) (Fig. 3). Upon stimulation by lipopolysaccharide (LPS), macrophages markedly increased production of dihydroceramide, which in turn inverted the topology of newly synthesized CCR5. While still reaching the cell surface, the N- and C-terminus of CCR5 with the inversed topology, which was designated as CCR5(B), is localized at cytosol and extracellular space, respectively (Denard et al. 2019) (Fig. 3).
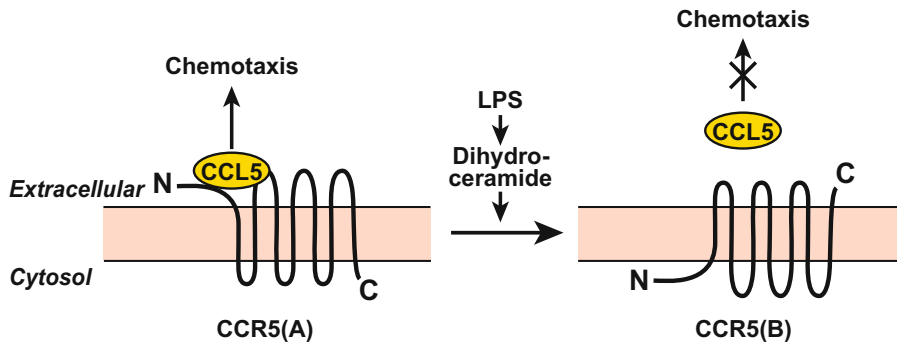
Fig. 3 **LPS inhibits chemotaxis through RAT of CCR5** In the absence of LPS, CCR5 adopts a topology consistent with that of GPCR (CCR5(A)) to guide macrophages migrating towards the gradient of its ligand, CCL5. LPS induces production of dihydroceramide, which in turn triggers topological inversion of CCR5 through RAT. CCR5 with the inverted topology (CCR5(B)) no longer binds CCL5, leading to inhibition of the chemotaxis reaction

CCR5(B) no longer functioned as a chemokine receptor, leading to inhibition of chemotaxis (Denard et al. 2019) (Fig. 3). The dihydroceramide-induced topological inversion of CCR5 was critical for LPS to prevent macrophages from migrating toward CCL5, as treatments inhibiting production of dihydroceramide relieved this inhibition of chemotaxis (Denard et al. 2019). These findings may explain the well-known observation that LPS-activated macrophages are insensitive to chemotaxis (Biswas and Lopez-Collazo 2009).

4 Concluding Remarks

The concept of translocation regulation has been proposed more than a decade ago (Hegde and Kang 2008). Ceramide-induced RAT of TM4SF20 and CCR5 discovered recently are the first two examples that this regulation indeed takes place in mammalian cells. Both TM4SF20 and CCR5 are polytopic transmembrane proteins, and their first transmembrane helix is translocated across membranes through the Type III insertion in the absence of excess ceramide. In contrast to the well-studied Type I and II insertions, the Type III insertion is much less characterized (Rapoport et al. 2017). Unlike the Type I and II insertions during which the newly synthesized peptide C-terminal to the transmembrane helix is pushed

through the translocation channel by ribosomes, sequence N-terminal to the transmembrane helix, which may have already been folded before initiation of the membrane translocation process, has to be pulled through the translocation channel during the Type III insertion. The driving force and unfolding mechanism that allows this sequence to be translocated through the Sec61 translocon remains obscure. Understanding the mechanism behind the Type III insertion may provide mechanistic insights into RAT of transmembrane proteins.

Perhaps the most important question raised by the discovery of RAT is the breadth of this regulatory mechanism. How many transmembrane proteins are regulated by RAT? Can RAT be triggered by other stimulations in addition to ceramide? The major obstacle to answer these questions is our limited knowledge on topology of transmembrane proteins in mammalian cells. According to UniProt, only less than 10% of transmembrane proteins expressed in mammalian cells have their topology experimentally defined. The topology of the rest of the transmembrane proteins is either unknown or predicted by sequence analysis, which may be plagued by errors. This problem is difficult to address by the currently available techniques, as they can only measure topology of one protein at a time, and often require overexpression of the protein tagged with an epitope, which may alter the topology of

the transmembrane protein. Thus, a proteome-wide approach capable of measuring topology of endogenous untagged transmembrane proteins expressed in mammalian cells globally is needed to systematically identify transmembrane proteins subjected to topological regulation.

Conflicts of Interest None.

References

Biswas SK, Lopez-Collazo E (2009) Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol 30:475–487

Bogdanov M, Dowhan W (1998) Phospholipid-assisted protein folding: phosphatidylethanolamine is required at a late step of the conformational maturation of the polytopic membrane protein lactose permease. EMBO J 17:5255–5264

Bogdanov M, Dowhan W, Vitrac H (2014) Lipids and topological rules governing membrane protein assembly. Biochem Biophys Acta 1843:1475–1488

Bogdanov M, Heacock PN, Dowhan W (2002) A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. EMBO J 21:2107–2116

Bogdanov M, Vitrac H, Dowhan W (2018) Flip-fopping membrane proteins: how the charge balance rule governs dynamic membrane protein topology. In: Geiger O (ed) Biogenesis of fatty acids, lipids and membranes. Cham, Springer International Publishing, pp 1–28

Brown M, Ye J, Rawson R, Goldstein J (2000) Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell 100:391–398

Chen Q, Denard B, Lee C-E, Han S, Ye JS, Ye J (2016) Inverting the topology of a transmembrane protein by regulating the translocation of the first transmembrane helix. Mol Cell 63:567–578

Chen Q, Lee C-E, Denard B, Ye J (2014) Sustained induction of collagen synthesis by TGF-β requires regulated intramembrane proteolysis of CREB3L1. PLoS One 9:e108528

Cui X, Cui M, Asada R, Kanemoto S, Saito A, Matsuhisa K, Kaneko M, Imaizumi K (2016) The androgen-induced protein AIbZIP facilitates proliferation of prostate cancer cells through downregulation of p21 expression. Sci Rep 6:37310

Denard B, Han S, Kim J, Ross EM, Ye J (2019) Regulating G protein-coupled receptors by topological inversion. elife 8:e40234

Denard B, Jiang S, Peng Y, Ye J (2018) CREB3L1 as a potential biomarker predicting response of triple negative breast cancer to doxorubicin-based chemotherapy. BMC Cancer 18:813

Denard B, Lee C, Ye J (2012) Doxorubicin blocks proliferation of cancer cells through proteolytic activation of CREB3L1. eLife 1. https://doi.org/10.7554/eLife.00090

Denard B, Pavia-Jimenez A, Chen W, Williams NS, Naina H, Collins R, Brugarolas J, Ye J (2015) Identification of CREB3L1 as a biomarker predicting doxorubicin treatment outcome. PLoS One 10:e0129233

Denard B, Seemann J, Chen Q, Gay A, Huang H, Chen Y, Ye J (2011) The membrane-bound transcription factor CREB3L1 is activated in response to virus infection to inhibit proliferation of virus-infected cells. Cell Host Microbe 10:65–74

Do H, Falcone D, Lin J, Andrews DW, Johnson AE (1996) The cotranslational integration of membrane proteins into the phospholipid bilayer is a multistep process. Cell 85:369–378

Dowhan W, Vitrac H, Bogdanov M (2019) Lipid-assisted membrane protein folding and topogenesis. Protein J 38:274–288

Duma L, Häussinger D, Rogowski M, Lusso P, Grzesiek S (2007) Recognition of RANTES by extracellular parts of the CCR5 receptor. J Mol Biol 365:1063–1075

Gewirtz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol 57:727–741

Görlich D, Hartmann E, Prehn S, Rapoport TA (1992) A protein of the endoplasmic reticulum involved early in polypeptide translocation. Nature 357:47–52

Guan XM, Kobilka TS, Kobilka BK (1992) Enhancement of membrane insertion and function in a type IIIb membrane protein following introduction of a cleavable signal peptide. J Biol Chem 267:21995–21998

Hegde RS, Kang S-W (2008) The concept of translocational regulation. J Cell Biol 182:225–232

Hijikata M, Kato N, Ootsuyama Y, Nakagawa M, Shimotohno K (1991) Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. Proc Natl Acad Sci U S A 88:5547–5551

Lee HC, Zhao YJ (2019) Resolving the topological enigma in Ca(2+) signaling by cyclic ADP-ribose and NAADP. J Biol Chem 294:19831–19843

Lodish H, Berk A, Kaiser C, Krieger M, Scott M, Bretscher A, Ploegh H, Matsudaira P (2007) Molecular cell biology, 6th edn. W. H. Freeman, New York

Lu Y, Turnbull IR, Bragin A, Carveth K, Verkman AS, Skach WR (2000) Reorientation of aquaporin-1 topology during maturation in the endoplasmic reticulum. Mol Biol Cell 11:2973–2985

Murakami T, Kondo S, Ogata M, Kanemoto S, Saito A, Wanaka A, Imaizumi K (2006) Cleavage of the membrane-bound transcription factor OASIS in response to endoplasmic reticulum stress. J Neurochem 96:1090–1100

Oppermann M (2004) Chemokine receptor CCR5: insights into structure, function, and regulation. Cell Signal 16:1201–1210

Patel AG, Kaufmann SH (2012) How does doxorubicin work? eLife 1. https://doi.org/10.7554/eLife.00387

Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8:785–786

Rapoport TA, Li L, Park E (2017) Structural and mechanistic insights into protein translocation. Annu Rev Cell Dev Biol 33:369–390

Voigt S, Jungnickel B, Hartmann E, Rapoport TA (1996) Signal sequence-dependent function of the TRAM protein during early phases of protein transport across the endoplasmic reticulum membrane. J Cell Biol 134:25–35

Von Heijne G (2006) Membrane-protein topology. Nat Rev Mol Cell Biol 7:909–918

Wang J, Kinch LN, Denard B, Lee C-E, Esmaeilzadeh Gharehdaghi E, Grishin N, Ye J (2019) Identification of residues critical for topology inversion of the transmembrane protein TM4SF20 through regulated alternative translocation. J Biol Chem 294:6054–6061

Winter E, Ponting CP (2002) TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains? Trends Biochem Sci 27:381–383

Xiao W, Liang Y, Que Y, Li J, Peng R, Xu B, Wen X, Zhao J, Guan Y, Zhang X (2019) Comparison of the MAID (AI) and CAV/IE regimens with the predictive value of cyclic AMP-responsive element-binding protein 3 like protein 1 (CREB3L1) in palliative chemotherapy for advanced soft-tissue sarcoma patients. J Cancer 10:3517–3525

Yang F, Teves SS, Kemp CJ, Henikoff S (2014) Doxorubicin, DNA torsion, and chromatin dynamics. Biochim Biophys Acta 1845:84–89

Ye J (2013) Regulated intramembrane proteolysis. In: Lennarz W, Lane D (eds) Encyclopedia of biological chemistry. Waltham, Academic, pp 50–55

Zimmermann R, Eyrisch S, Ahmad M, Helms V (2011) Protein translocation across the ER membrane. Biochim Biophys Acta 1808:912–924